Purification and properties of a phytate-degrading enzyme produced by Enterobacter sakazakii ASUIA279.

ABSTRACT

An extracellular phytate-degrading enzyme produced by Enterobacter sakazakii ASUIA279 was purified to homogeneity using FPLC anion exchange chromatography and gel filtration. The enzyme was purified about 66-fold with a recovery of 27%. Its molecular mass was estimated to be 43 kDa by SDS-PAGE. The Michaelis constant (KM) and turnover number (kcat) for sodium phytate at pH 5.0 and 50°C were calculated from the Lineweaver-Burk plot to be 760 µM and 4.14s-1, respectively. The enzyme showed narrow substrate specificity and not phytate, but GTP was dephosphorylated with the highest relative rate of hydrolysis. However, according to the kcat/KM values, phytate was concluded to be the in vivo substrate of the enzyme. Optimal activity was determined at pH 4.5 and 45-55°C. The enzyme was strongly inhibited by Fe3+, Cu2+, Zn2+, molybdate, vanadate, fluoride and phosphate (1 mM).

Keyword: Enterobacter sakazakii; Phytate-degrading enzyme; Phytate; Purification.